S/N 09/125,953 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

OFFICIAL

Applicant:	Fostad et al.	Examiner:	B. Sisson
Serial No.:	09/125,953	Group Art Unit:	1655
Filed:	December 10, 1998	Docket No.:	7885.56USWO
Title:	IMMUNO-MAGNETIC CELL SEPARATION USED IN IDENTIFICATION OF GENES ASSOCIATED WITH SITE-PREFERENCED CANCER METHASTASIS FORMATION		

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In response to the Office Action of August 9, 2000, applicant provides the following amendments and remarks.

In the Claims

Please cancel claims 10 and 11.

Please amend claims 2-9 as follows:

a1

2. (Amended) Method according to claim [1] 12, [characterized in that] wherein the [used] first and second tissue target cells are malignant cells obtained from solid primary or recurrent tumors; and/or from metastases from such tumors to lymph nodes; and/or blood; and/or bone marrow; and or blood tissue; and/or liver; and/or lungs; and/or central nervous system; and/or malignant pleural effusions and ascites; urine; and/or cerebral spinal fluid; and or other organ sites.

subF1

3. (Amended) Method according to claim [1] 12, [characterized in that] wherein the first and second tissue target cells are malignant cells [are] isolated from single cell suspensions prepared from solid tumor manifestations; and/or from mononuclear cell fractions obtained from bone marrow or blood samples; and/or from cells present in other bodily fluids.

4. (Amended) Method according to claim [1] 12, [characterized in that] wherein the first and second tissue target cells are malignant cells [used are] cultured *in virto* cultivated human tumor cells; and/or human tumor cells grown in specific tissues in immunodeficient animals; and/or experimental human tumor metastases in such animals.

5. (Amended) Method according to claim [1] 12, [characterized in that] wherein RNA and DNA are extracted from the isolated cells.

6. (Amended) Method according to claim 5, [characterized in that] wherein the extracted nucleic acids are used for gene cloning purposes.

7. (Amended) Method according to claim [1] 12, [characterized in that the said gene cloning method is the] wherein the identification of differential gene expression is determined by differential display or [the] subtractive hybridization [approaches], or any other procedure that can be used to identify genes with differential expression.

8. (Amended) Method according to claim 7, [characterized in that] wherein amplified cDNAs are obtained from malignant cells selected from [different sites are studied and] the first and second tissues and are compared on sequencing gels, and [where those] wherein interesting site-specific or site-preferenced patterns are sequenced and identified.

9. (Amended) Method according to claim 8, [characterized in that] wherein the expression pattern of identified gene sequences are studied on material obtained from [all] two or more relevant tumor sites.

Please add the following new claim:

12. (New) Method for identifying genes differentially expressed between cells isolated from different tissues, the method comprising:

A. detecting target cells from a first and a second tissue;

B. obtaining nearly 100% specific target cells by repeatedly immunomagnetically isolating said first and second tissue target cells;

B. determining levels of mRNA expression within said first and second tissue target cells;

C. comparing the levels of mRNA expression in said first and second tissue target cells; and

D. identifying the genes differentially expressed between said first and second tissue target cells based on the comparison of the mRNA expression levels,

wherein at least one of said first and second tissue target cells are tumor cells.

Remarks

No new matter has been added. Claims 10 and 11 have been canceled. Claim 12 has been added. Claim 12 more clearly sets forth the method of claim 1. Claims 2-9 have been amended to depend from claim 12 and to more clearly define the invention.

Anticipation Rejection

Claims 1-9 have been rejected under 35 U.S.C. 102(b) as allegedly being anticipated by Hoifodt et al. Applicants respectfully traverse the rejection.

Hoifodt et al. does not disclose the presently claimed invention. Hoifodtet al. does not disclose gene cloning to study the differences in mRNA expression levels by comparison.

In the Examiner's August 9, 2000 Communication, it was stated that "[t]he aspect of isolating, culturing, and studying cancer cells is considered to meet the limitation of a clonal cell population as metastatic cancer is considered to have spread from a single tumor that arose from a single cell."

However, cell cloning and the claimed gene cloning are totally different processes. Furthermore, it is not presently considered clear to what extent a primary tumor arises from a single cell. The tumor can consist of multiple subpopulations of cells with different

chartacteristics. One skilled in the art knows that the expression patterns of primary tumors and metastatic tumors often are distinct (see Fostad et al. Cell. Biochem. 56:23-29, 1994, enclosed). Accordingly, it is now accepted that if a primary tumor had arisen from one cell, all the cells should be identical. This is not the case.

When the present application was filed, such differences at the genetic level were difficult to assess. Moreover, attempts to identify previously unknown genes involved in such differences were hampered by the major difficulties in obtaining pure tumor cell populations. The presently claimed invention overcomes such difficulties, allowing one to study the differences in mRNA expression levels by comparison.

The cited reference does not teach cell cloning, and if it did, it would not teach the presently claimed invention. Removal of the rejection is respectfully requested.

Applicants respectfully assert that the instant claims are in a condition for allowance, and notice to that effect is earnestly solicited.

If the Examiner has any questions regarding the foregoing, it is respectfully requested that he call the undersigned.

Respectfully Submitted,

MERCHANT & GOULD P.C
P.O. BOX 2903
Minneapolis, MN 55402-0903
612-371-5265

John J. Gresens
Reg. No. 33,112
JJG/KMC/pjr